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The effect of low pH on the glycitein–BSA conjugate interaction with specific antiserum: Competitive inhibition study using surface plasmon resonance technique

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ABSTRACT

Competitive inhibition serological assay for detection of the phytoestrogen glycitein (Glyc) was developed using surface plasmon resonance (SPR) technique with protein conjugates and polyclonal antibodies initially designed for the enzyme-linked immunosorbent assays (ELISA). The efficiency of the approach to the quantification of the soy isoflavone glycitein in water was investigated using the competitive reaction of analyte (free Glyc)and immobilized Glyc–BSA-conjugate with polyclonal antibodies. It was shown that the efficiency to detect Glyc drastically depends on the pH level of the probe solution. With the decrease in pH from 7.4 to 4.0, (i) the affinity of the specific reaction increases and (ii) the level of unspecific sorption becomes saturated. Non-specific adsorption to a SPR sensor surface obscures the specific component and shaded specific response at higher pH (6.0–7.4) when used serum for the quantification of specific analytes. The standard curves obtained in acidic solutions (pH 4–5) indicate that the linear part of the dependence completely covers the range between detection limit (0.1 μg/ml) and Glyc solubility in water (0.9 μg/ml). The difference in SPR- and ELISA-based analytical protocols as well as the requirements for increasing the efficiency in quantitative SPR analysis using purified antibodies is discussed.

1. Introduction

Current advances in the detection of different chemical and biological substances, such as xenobiotics, toxins, drugs or micronutrients is becoming possible mainly thanks to successful progress in scientific instrumentation and the development of various methods for direct analysis of intermolecular interaction. Biosensor systems based on optoelectronic and acoustic transducers have occupied a special position in this area. They make it possible to design devices that generate an informative signal in real time without any additional labelling of target molecules [1]. Analytical approaches based on surface plasmon resonance (SPR) transducers provide a classical example of biosensor methods suitable for a wide range of both fundamental and practical applications. In addition to the qualitative direct detection techniques, the SPR systems can also be applied to the development of quantitative assays in a competitive mode [2,3] that open the way to overcome the principal limitations of SPR systems for low-molecular weight analytes [4,5]. The immunological analytical techniques based on the competitive inhibition analysis for detection of low-molecular chemical species such as toxins [6], hormones [7], pesticides [8], and biologically active compounds [9,10] are the classical examples of such applications.

For the development of an SPR-based version of competitive immunoassays, it is promising to consider using molecular biorecognizing systems (hapten–protein conjugates and corresponding specific antiserum) that have already demonstrated their potentialities in user-proven and validated conventional techniques. In particular, the use of biochemical reagents originally designed for the development of a classical enzyme-linked immunosorbent assay (ELISA) allows a considerable reduction in the time required for the development of a viable SPR diagnostic system. To verify the adequacy of such an approach, experimental investigations of interactions between a biological receptor (antibody) and a specific analyte (compound to be detected) under conditions of competitive binding on the surface (immobilized conjugate with analyte analogue) and in bulk (analyte) are needed. The reason for such research is the difference in both a signal generation technology

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Table 1
Comparison of analytical protocols based on ELISA and SPR.

Detection method	ELISA	SPR
Analytical steps	In general, consecutive reactions, two steps	In general, direct analysis, single step
Surface material/profile	Normally polymer matrix/rough, disordered	Metal (gold, silver etc.)/smooth, short-range ordered
Informative signal source	Sum of single events: additive emissions from isolated labeled secondary antibodies	"Optical thickness": proportional to the thickness and the effective refractive index of interfacial architecture
Selectivity enhancement	Refinement of response owing to high specificity of secondary antibodies	Data corruption by the adsorption of non-specific components on the supporting matrix

and an interfacial sensing environment specific for ELISA and SPR chips (Table 1).

In this study the phytoestrogen glycitein (Glyc) was chosen as a model analyte (Fig. 1). It is a major isoflavone of soy germ (about 40% total isoflavone content) [11]. Glyc is present in soy-based foodstuffs, beverages, and neutraceutical supplements. It is currently used as a functional ingredient to enrich foods with isoflavones in order to improve metabolic parameters in postmenopausal women as well as in alternative medicine for the treatment of breast cancer patients [12]. However, the role of isoflavones in breast cancer has become controversial because, in contrast to the possible beneficial effects, some recent data suggest that isoflavones may stimulate the growth of sex-hormone-dependent tumors [13]. Thus, the importance of developing different quick methods for detection of natural isoflavones, in particular Glyc, seems to be beyond doubt.

The purpose of the present study was to explore the utilization of biochemical reagents originally designed for the development of a classical enzyme-linked immunosorbent assay [14] as the recognition interfacial architecture for detection of the phytoestrogen

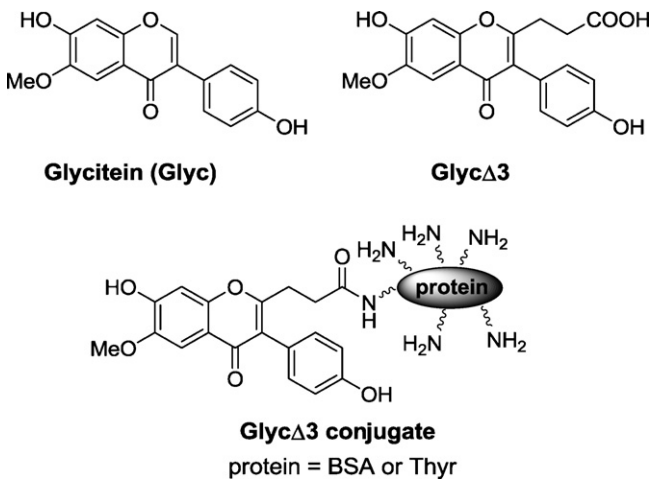

Fig. 1. Chemical structure of glycitein, corresponding hapten and protein conjugates used.

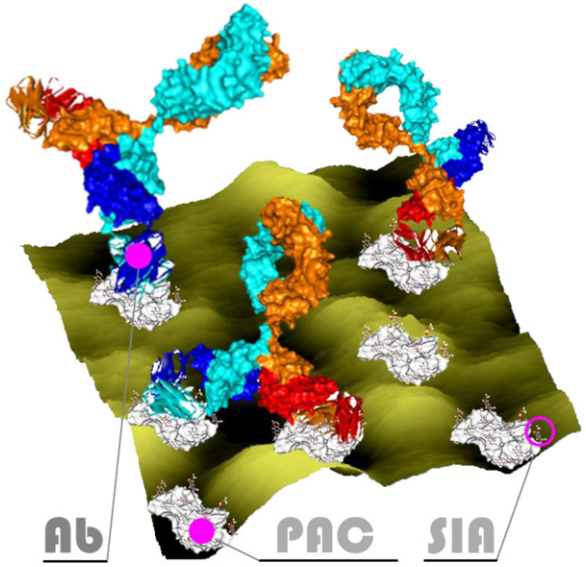

Fig. 2. Schematic presentation of the inhibitor-type competitive analysis. A certain amount of a biological receptor (Ab) is added to the probe containing the analyte to be detected. Then, the mixture is placed over the sensitive element on which analyte analogue (protein–analyte conjugate, PAC) is immobilized. The method determines the quantity of antibodies that did not react with analytes in the solution and thereby are capable of binding to the surface immobilized analyte (SIA). Their quantity depends on the relation between antibody concentration, the number of binding sites at the surface and analyte concentration.

glycitein by SPR diagnostic system.

The paper is organized as follows. At the beginning the methodological aspects of competitive inhibitor analysis specific for SPR transducers are discussed. Then the experimental data that demonstrate the particularities of biochemical reactions occurring on the SPR transducer surface are presented. After this, an analysis of Glyc detection efficiency at various pH levels is given, along with discussion of the standard curves of competitive analysis obtained. Finally, some approaches for developing the SPR-based methodology, as well as some recommendations concerning application of the ELISA biochemical sets for setting up SPR protocols, are discussed.

2. Methodological features of application of SPR sensors for competitive analysis conditions

In indirect methods of analysis, two types of ligands, namely, the antigen Ag (in our case the free analyte), and its immobilized analogue (in our case, an analyte–protein conjugate) compete for the binding process with the same biological receptor (in our case specific antibodies Ab), as presented in Fig. 2. According to the principles of the traditional competitive analysis in the steady-state regime the surface concentration of bounded receptors (antibodies) Γ is given by the Morgan–Mercer–Flodin equation, or the logistic curve [15]:

$$\Gamma = \frac{(V/S)^{\bullet} C_{Ab}}{1 + ((C_{Ag}/\Gamma_{Ag})^{\bullet}(V/S))^{p}} \quad (1)$$

here p is the order of reaction with respect to the antigen (an effective quantity that carries information on the mechanism of processes occurring in the system: the bigger the parameter p value, the greater extent to which the processes of univalent analyte binding to a bidentate antibody takes place concurrently); C_{Ab} is the initial concentration of antibodies Ab in the solution; C_{Ag} and Γ_{Ag} are the concentration of antigen in the solution and concentration of its immobilized analogue on the surface, respectively; V and S are cell volume and sensitive surface area, respectively. Eq. (1)

also illustrates directly the linear dependence of the response on the antibody concentration and possibility to optimize both the dynamic range and minimal detectable value by variations of C_{Ab} and Γ_{Ag}. A number of recommendations of practical importance that can be used for purposeful development and optimization of applied procedures are able to be obtained [15].

To determine how the concentration of surface-bound complexes depends on the amount of analyte in the solution it is reasonable to apply the SPR method. It is known that at linear approach [5] a change of adsorbed mass ($\Delta M = \Gamma \cdot S$) on the surface of a physical transducer may be related to the SPR minimum position shift (ΔSPR) [16,17]:

$$\Delta M = \chi^{\bullet} \Delta SPR \quad (2)$$

where χ is an instrument constant that is to be determined in accordance with [18]. Since SPR transducers are inherently nonselective, ΔM is an effective quantity determined by a superposition of various processes at the interface, leading to a change in the refractive index in the immediate vicinity of the surface. As a result of this, the effective mass of the substance on the surface of the transducer will be determined by statistical averaging of individual specific (superscript Ab) and unspecific (superscript *i*, i.e. any molecule from the probe that can adsorb on the surface with the exception of Ab) reactions and is written as follows:

$$\Delta M = N^{\bullet} \omega^{Ab} \bullet P^{Ab} \bullet \delta^{Ab} + \sum_{i \neq Ab} \omega^{i} \bullet n^{i} \quad (3)$$

here ω is the molecular mass of the adsorbate, $N = \text{Const} \cdot S/S^{*}$ is the maximal number of receptor molecules capable of binding to the surface; S and S^{*} are the areas of the transducer surface and projection of a macromolecular receptor Ab onto this surface, respectively, and the factor Const ≈ 0.7 is due to the effects of stochastic packaging [16,17]. The parameter n^{i} is the number of molecules *i* with molecular weight ω^{i} that can be adsorbed on the surface. The second term on the right-hand side of Eq. (3) reflects the process of nonspecific adsorption (non-Ab) on the surface. Eq. (3) illustrates the complicated character of surface covering formation, especially in case of the presence of wide diversity nonspecific components *i*. The number of Ab molecules bound to the surface is determined by the molecular state (conformation, charge state, etc.) of the grafted ligand analogue (δ^{Ab}) and by the distribution of such ligands over the surface (P^{Ab}). So, for SPR the proceeding of surface reactions is dependent not only on the recognition properties of biological components but as well on the spatial orientation and distribution of interacted partners on the surface.

Keeping in mind that we are using biochemical compounds (Fig. 1) initially designed for ELISA protocols it is reasonably to highlight the difference in conditions specific for reactions occurring on the SPR sensor surfaces and in ELISA [1,15,18]. In ELISA immobilization of the biological components proceeds on modified hydrophobic polymer surface, routinely transparent polysterene. According to AFM imaging data the topography of such material surface represents lengthy ridges with the vertical dimensions from several to ten nanometers with various extent of parallelism and with alternating unstructured areas [19]. Such surface relief prevents the formation of dense interfacial architectures with either short- or long-range order of immobilized compounds. As a result, the interference between the nearby molecules on the surface is insignificant and their interactions with the complementary partner (analyte specific primary antibody) from solution are not dependent on the arrangement of recognition centers. Using the high specific (against the Fc fragment of primary antibodies) labeled secondary antibodies not only generate the informative signal but also exclude the signal from adsorbed non-specific (non-Ab) serum components.

At the same time, in SPR method immobilization of the biological component is made on the gold smooth surface. The topography of such surfaces is characterized by a low root-mean-square roughness, and absolute differences of surface irregularities are from parts to few nanometers, that less than characteristic dimension of the used proteins [18]. As a result the formation of high-organized two-dimensional structures is natural process on such surface, and interference of molecules in a layer is very significant [15–17]. Moreover, in line with Eq.3 the informative signal is proportional to the total mass (both specific and non-specific adsorbed compounds) on the surface.

3. Experimental

Bovine serum albumine (BSA) and bovine thyroglobulin (Thyr) were purchased from Sigma Aldrich (Saint Quentin Fallavier, France). Glyc was used as standard in the assay, a hapten of Glyc (GlycΔ3), bearing a spacer arm of three carbon atoms (Δ3) as well as GlycΔ3–BSA and GlycΔ3–Thyr conjugates were prepared as already described (Fig. 1) [14]. Rabbit antiserum produced against GlycΔ3–BSA was lyophilized, stored at −20 °C and dissolved at 1 mg/ml in distilled water before utilization. The protein conjugate (1 mg/ml) was dissolved in phosphate buffer (PBS, 0.01 M, pH = 7.4, 0.9% NaCl) and kept at −20 °C.

Because of the very low water solubility of Glyc, a special procedure was used to obtain the stock solution of Glyc as well to determine its concentration. At first 1.5 mg Glyc and 1.5 ml PBS were mixed for an hour at a temperature of 60 ± 3 °C. Then the volume of the turbid colloidal solution obtained was brought to 15 ml PBS, filtered out through a paper filter, packed up 400 μl and kept at +4 °C until application. When needed, the stock solution of Glyc in PBS was diluted down to the required concentration with the corresponding buffer.

Taking into account that the true concentration of Glyc in water remains unknown in such a procedure, calibrating solutions were prepared with concentrations of $[C_{cal}] = 10$ μg/ml of Glyc in the 50/50, v/v mixture of water and ethanol. As variations of the absorption coefficient of the Glyc low-energy band (wavelength of about 320 nm) for different solvents are small [20,21], the water solutions were standardized using the spectrophotometric technique (Spectrophotometer Unico, USA) relative to those in aqueous–alcoholic mixture (see Fig. 3a). The stock solution concentration obtained in such way was $[C_{est}] = 0.9$ μg/ml (solubility limit of Glyc in distilled water). This value was used for the calculation of further dilutions.

The electronic absorption spectra of conjugate GlycΔ3–BSA (concentration of 0.125 mg/ml) demonstrate both the BSA absorption band and the low-energy absorption band of protein-bound Glyc that indicate stability of GlycΔ3–BSA composition (Fig. 3b). As compared to the spectrum of Glyc in solution, the immobilized ligand is characterized by somewhat broadened absorption bands. This seems to be caused by cumulative effect of molecules on the protein surface (according to MALDI data, there are about 20 ligand molecules per a protein molecule [14]).

We performed our investigations using an SPR spectrometer "BioHelper" with angular scanning and a GaAs laser as source of excitation (at the wavelength λ = 650 nm) that was developed at the V. Lashkaryov Institute of Semiconductor Physics of the National Academy of Sciences of Ukraine [22]. Glass plates ($n = 1.61$) with a 50 nm gold layer evaporated through a chromium adhesive interlayer (1–1.5 nm) were fixed to a supporting glass prism ($n = 1.61$). Optical contact was ensured with an immersion liquid (polyphenyl ether, $n = 1.6$). The measurements were performed without mixing, in the static mode without sample flow; the volume of an open cell was 400 μl. To determine the position of minimum in the SPR curve the angular dependence has been approximated by

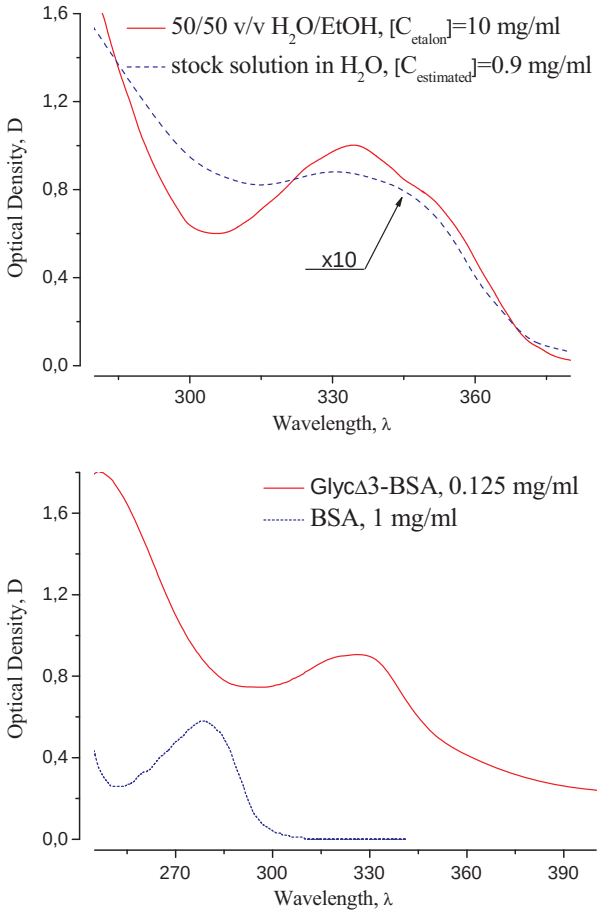

Fig. 3. Absorption spectra of Glyc (a, left) in water (dashed curve) and aqueous–alcoholic mixture (full curve), as well as conjugate GlycΔ3–BSA (b, right, full curve) and BSA (b, dashed curve).

third-order polynomial function [22].

The research protocol involved the following main steps. The device cell was filled with distilled water, and the base line was measured. Then the water was replaced (for 5 min) with a freshly prepared mixture HCl(37%)/H_2O_2(30%)/H_2O (3/3/15, v/v/v) to remove organic contaminants [23]. After this, the mixture was replaced with water. Three consecutive short-term water/PBS replacements ensured sufficient surface washing of the residual acid–peroxide mixture, as well as serving as reference mark (the calibrating variation of the refractive indices of water and PBS is about 0.004) when comparing experiments performed on different SPR chips.

After the last water/buffer replacement, the cell was filled with conjugate solution in PBS (50 μg/ml) for 10 min, and then washed with PBS and water. After this, the cell was filled with a buffer having the required pH value (4.0, 5.0 and 6.0 – citrate buffer, 7.4 – PBS). This buffer, in turn, was replaced with the solution under investigation (antibodies, or a mixture of antibodies and analyte in the same buffer, pre-incubated at room temperature for 30 min). After measurement for 10 min, the cell was washed with the buffer again. To estimate the level of non-specific binding of probe components on the BSA matrix special chips covered by pure BSA (instead of GlycΔ3–BSA conjugate) were used.

The data from the initial portion of the kinetic curve (10 min, time required for attainment of saturation was about 30 min. in most cases) were used for analysis to decrease an input from additional binding of nonspecific compounds. To approximate the curves and linear dependences, the procedures of OriginPro 7.5 (OriginLab Corporation) were applied in line with the classical version of competitive analysis (Eq. (1)) for a cell with a constant volume of the probe without a flow of reagents[15].

The analysis of SPR kinetic was performed in the frame of the model that takes into account heterogeneous processes at the interface, using the stretched exponential function [24,25]:

$$\Delta SPR(t) = \Delta SPR_{max} \bullet \left(1 - \exp\left(-\left(\frac{t}{\tau}\right)^{\beta}\right)\right) \quad (4)$$

where ΔSPR_{max} is the saturation level of the response, τ is a characteristic time and β is a parameter that has a value that depends on the particular interfacial processes involved. Classical example is the interfacial processes with $\beta = 1$ that corresponds to the classical Langmuir model. In cases with $\beta = 0.5$, the limitation of the diffusion occurred near or on the surface.

4. Results and discussion

Taking into account the different environment for the proceeding of the analytical reactions in SPR and ELISA, it is necessary initially to check if the functional properties of protein complexes on the gold surface of the SPR chips are retained. To this end, firstly the ability of two protein conjugates, namely GlycΔ3–BSA (~73 kDa) and GlycΔ3–Thyr (~670 kDa) [14], was tested to bind the specific components of polyclonal serum. Both conjugates demonstrated high functionality when detecting Glyc by ELISA [14]. However, the Thyr-based conjugate did not demonstrate an ability to bind specific immunoglobulins when one has been immobilized on the gold surface of SPR chips (data not shown). It was not used in further experiments. Contrarily, the GlycΔ3–BSA conjugate immobilized on the surface retained its ability to bind the specific antibodies. This was tested in experiments with a series of dilutions (1:20–1:8000, data not shown).

Fig. 4 presents the typical kinetic curves that illustrate how serum binds to both GlycΔ3–BSA- and BSA-modified surfaces at different pH levels of the probe. The SPR responses were also recorded in the presence of maximal Glyc concentration (0.9 μg/ml, solubility limit of Glyc in water). Fig. 5 presents the SPR sensor responses after incubation for 10 min followed by washing with the corresponding buffer to remove weakly bound non specific serum components (that is practically absent in all the cases considered; corresponding data not shown). One should note that the decrease in pH value from 7.4 down to 4.0 leads to a growth (by a factor of 3–5) in the number of surface-bound compounds. In this case, such variations occur for both specific and nonspecific binding. Only when the pH level drops below 5.0, will the specific response from the sensor become more pronounced as compared with that of the nonspecific part of the polyclonal serum (Fig. 5). Thus, in the present case the efficiency of detecting a specific reaction (Glyc–Ab interactions) is restricted by the adsorption of other serum components on the surface. Similar behaviour for all curves at pH 6.0 and 7.4 is determined largely by the unspecific interfacial reaction carrying insignificant information (second term in Eq. (3)). The protein background that is present in the serum obscures the specific component and shaded specific response. So, the specific reaction can be detected, if the effect of the background is reduced. As can be seen from Figs. 4 and 5 specific reactions can be detected more clearly at a low pH level owing to the saturation of nonspecific binding (on the BSA surface) in acidic solutions. At high pH levels unspecific adsorption is dominant. In fact, in this case competitive analysis cannot be applied.

Some additional information can be extracted by using the approximation of the kinetic curves presented in Fig. 4 by stretched exponential function in accordance with Eq. (4). Interestingly, a good fit to the kinetics of the absorption process on the pure BSA

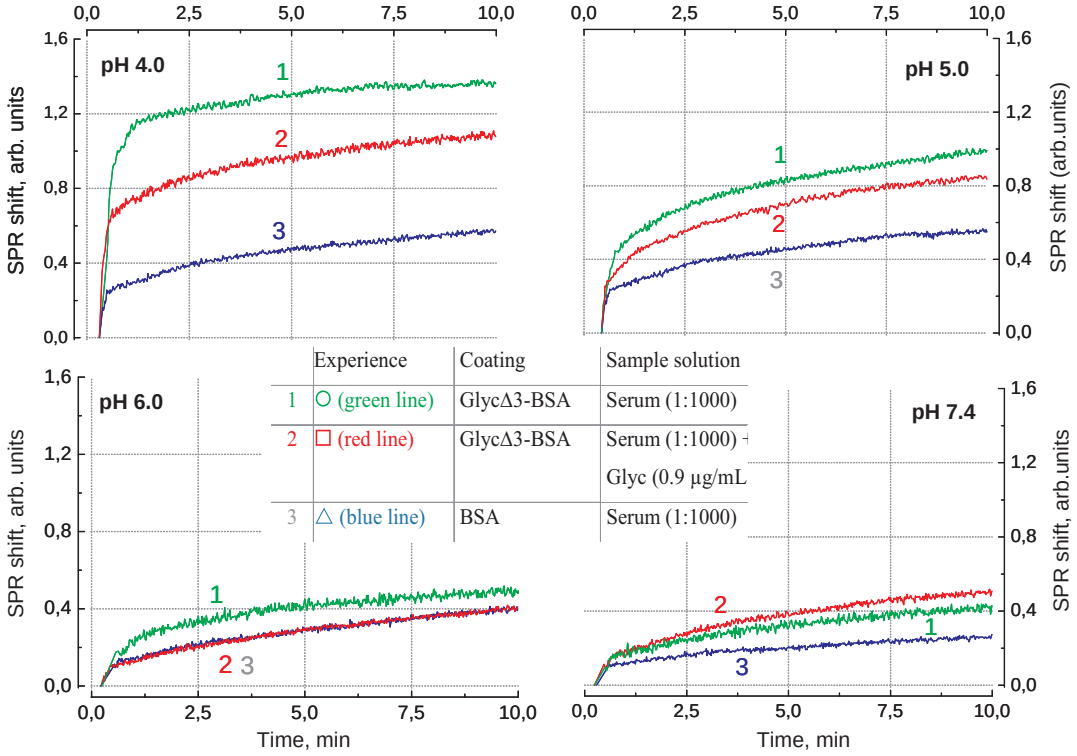

Fig. 4. Typical SPR responses at different pH levels. Zero level corresponds to the SPR response in working buffer. Washing the SPR chips with the working buffer after probe exposure do not change the SPR response owing to practically absent weakly bound nonspecific serum components in all the cases considered (data not shown).

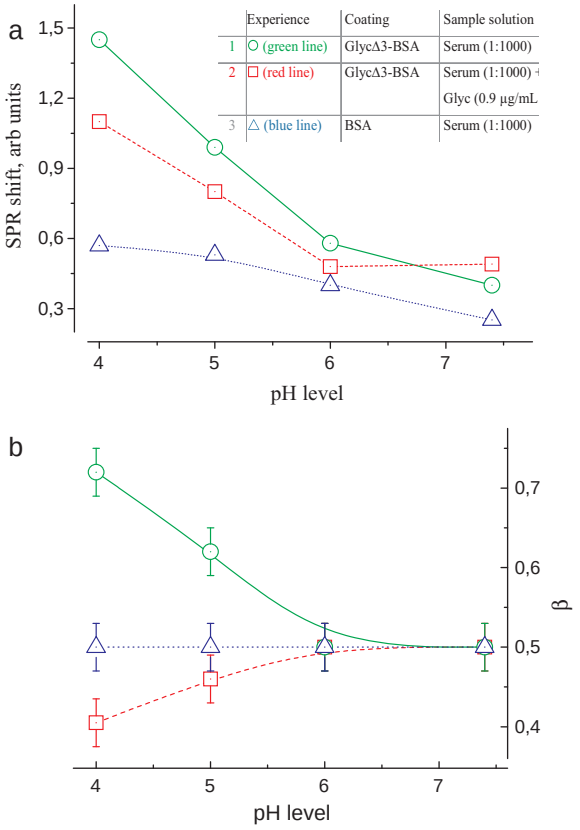

Fig. 5. Quasi-stationary SPR responses (10 min after the start of exposure the sample to the SPR chip) and calculated values of exponent power β (b) obtained by approximation the experimental curves (Fig. 4) using stretched exponential function.

surface is obtained if a stretched exponent with $\beta = 0.5$ is used. This points to the absence of any normalizing process in this case and classical diffusion mechanism dominants for generally nonspecific surface binding. Totally different situation has been observed for both serum and serum–Glyc probes at low pH. The kinetics of the specific IgG containing serum interaction followed the absorption model for the selective interaction, which is influenced by diffusion processes near the surface. A βvalue of 0.62 ± 0.03 (pH 5.0) and 0.72 ± 0.03 (pH 4.0) was obtained for the stretched exponential function that may suggest the increasing the specificity of the interfacial reactions with decreasing the pH. Some additional deceleration of interacting molecules near the surface in the presence of Glyc (β value of 0.46 ± 0.03 (pH 5.0) and 0.41 ± 0.03 (pH 4.0)) means that some additional ordering is required for interfacial architecture formation when the average distance between the neighboring Glyc centers is about parts of nm and possible readsorption processes take place.

The observation that specific reaction efficiency against low-molecular antigens (haptens) increases as pH value goes down seems rather amazing, because the structure of IgG recognizing sites is conservative and does not stimulate antigen variability when conditions change near the physiological conditions at least. Indeed, in the case of proteins, when external conditions change (pH, temperature, ionic force, solvent, etc.), either the preservation of biological activity is assured by the conformational stability provided by the secondary structure at macromolecular level or the system loses its biological functionality. It is obvious that, contrary to "self-adapting" protein superstructures that can maintain the structural particularity of important elements, the low-molecular compounds do not possess such properties. Variations in external conditions lead to a change of their conformation, assembling, orientation on the surface and electronic structure. Taking into account the high specificity of IgG–antigen binding, even a small variation of ligand conformation can change the affinity of immunospecific reaction, as demonstrated earlier for

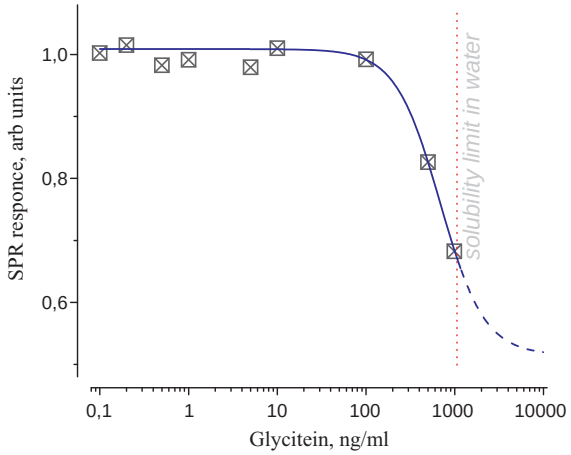

Fig. 6. Stationary curves for response of an SPR sensor with immobilized GlycΔ3–BSA at different concentrations of Glyc in citrate buffer (pH = 5.0) and constant concentration of the antibodies against GlycΔ3–BSA (serum dilution of 1:1000). The best approximation according to Eq. (1) is shown with full curve (the order of reaction p is above 1.7).

sulfamethoxazole in acidic solutions [15]. Formation of intramolecular complexes of analyte molecules as well can affect the efficiency of immunological methods. Immobilization of analyte analogues (hapten GlycΔ3) on the protein surface preserve the interactions between neighbour molecules. As a result the specific IgG was generated against fragments of monomeric analyte. At the same time, in the solution the majority of isoflavones have a tendency to dimerize [26,27]. In this case the specific site of monomeric analyte can be blocked by the second molecule. The equilibrium between monomeric and dimeric forms can be influenced by pH owing to changing in electrostatic interactions in dimeric assemble.

The curve that corresponds to serum adsorption on the surface of unmodified BSA matrix (blue line, Fig. 5) reveals the level of nonspecific sorption. Increase in, and saturation of, adsorption capacity at low pH levels on the BSA surface seems to be caused by diminishing the electrostatic interactions (normally resulting in an increase in protein aggregation) when approaching their isoelectric point. The pI value for BSA is about 4.7–4.8, so pH change leads to a decrease in the number of nonspecifically bound proteins because of electrostatic repulsion.

To illustrate the possibility of competitive analysis of Glyc at low pH, a standard curve plotted from the data on responses after a 10 min measurement of the Glyc solutions (with known concentrations) is presented in Fig. 6. It should be noted that close-to-linear dependence of the sensor information signal (0.1–0.9 maximal response) on Glyc concentration completely covers the range that is determined by the minimal concentration (0.1 μg/ml) and Glyc solubility in water (about 0.9 μg/ml). It can be seen as well that Eq. (1) gives an adequate description for experimental dependence over the whole range of concentrations used. The approximation of the experimental data presented in Fig. 6 by Eq. (1) gives the effective order p as being above 1.7. The value p indicates a possible parallel binding of antigens by both F_{ab} regions of antibody, and thus the molecularity of the interfacial reaction that, in the present case, is closely to the bidentate binding.

5. Concluding remarks

The development of various detection techniques for phytoestrogens that are present in soya and soybean products is of importance for the inspection of food quality and is specified by the European norms and regulations [28]. The tools for solving the problem of quality control are based mostly on the application of ELISA competitive analysis or HPLC methods. The possibility of applying similar biological sets in other modern techniques, such as SPR or similar, opens alternative ways for solving specific tasks, especially when analysis time is limited, the cost is high-priced, or analysis has to be performed in the field. At the same time, successful realization of the transducer based-version of competitive analysis strongly depends on the possibility to prevent the non-specific adsorption on the sensor surface by optimization of the analytical protocol (e.g. pH level etc.). Alternative way is applying purified antibodies instead of polyclonal serum containing a broad non-specific protein background that results from the difference in mechanisms of informative signal generation in SPR and ELISA. Only the specificity of secondary tagged antibodies towards the primary ones (obtained from serum) plays a role in classical ELISA, and their selectivity can be maximized. The situation is different in SPR-based tests. In this case, an information signal of the SPR transducer is formed as a superposition of responses from all objects (of both specific (Ab) and non-specific (non-Ab) nature) that are in the immediate vicinity of the surface, i.e. the signal is determined by the adsorption properties of both the receptor centers (immobilized analogue of Glyc) and the supporting matrix (BSA). Additional possibility in control of non-specific reactions can be realized using sensitive surfaces based on adsorptive-inert self-assembling architectures (e.g. mixed monolayers instead of BSA matrix), make it possible to form functional interfacial architectures with sustained performance and long shelf life [17]. Thus, the development of SPR-sensors with an inert matrix and a spatially organized system of surface receptor centers of a preset nature is the principal direction for the development of the competitive analytical tools based on SPR technique. Taking into account the considerable reduction of cost (no tagged and revealing reagents are needed), fast analysis of the information about the evolution of the reaction in the real-time mode, the possibility to tune the range of concentrations to be determined and the capacity of current microarray technologies thereon abundantly evidence that using of the validated biochemical kits for ELISA or similar technologies is the reasonable way for the development of compact-size fast-operating SPR-based test systems for practical applications.

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